Acta Pædiatrica ISSN 0803–5253

REGULAR ARTICLE

Pasteurization of mother's own milk reduces fat absorption and growth in preterm infants

Y Andersson[1], K Sävman[2], L Bläckberg[3], O Hernell[1] (olle.hernell@pediatri.umu.se)

1.Department of Clinical Sciences, Pediatrics, Umeå University, S-901 87 Umeå, Sweden
2.Perinatal center, The Queen Silvia Children's Hospital, S-416 85 Gothenburg, Sweden
3.Department of Medical Biosciences, Physiological Chemistry, Umeå University, S-901 87 Umeå, Sweden

Keywords
Bile salt-stimulated lipase, Fat absorption, Human milk, Pasteurization, Preterm infant

Correspondence
Prof. Olle Hernell, Department of Clinical Sciences, Pediatrics, Umeå University, S-901 87 Umeå, Sweden.
Tel: +46(0)907852101 |
Email: olle.hernell@pediatri.umu.se

Received
5 May 2007; revised 2 July 2007; accepted 6 July 2007.

DOI:10.1111/j.1651-2227.2007.00450.x

Abstract

Aim: A randomized study was conducted to evaluate whether pasteurized milk (Holder pasteurization 62.5°C, 30 min) reduces fat absorption and growth in preterm infants.

Methods: Preterm infants (825–1325 g) born with gestational age ≤30 weeks were randomized into two groups, of which one started with pasteurized own mother's milk for 1 week and continued with raw milk the following week, and a second group was fed in reverse order. By using this design the infants served as their own controls. At the end of each week, a 72-h fat balance was performed and growth was monitored.

Results: We found, on an average, 17% higher fat absorption with raw as compared to pasteurized milk. Infants gained more weight and linear growth assessed as knee–heel length was also greater during the week they were fed raw milk as compared to the week they were fed pasteurized milk.

Conclusion: Feeding preterm infants pasteurized as compared to raw own mother's milk reduced fat absorption. When the infants were fed raw milk, they gained more in knee–heel length compared to when they were fed pasteurized milk.

INTRODUCTION

Pasteurization of donor breast milk is routine in many neonatal intensive care units (NICUs) to prevent transmission of potential pathogens. At some NICUs, it has become routine also to pasteurize own mother's milk for very low birth weight (VLBW) infants. Holder pasteurization (62.5°C, 30 min) is the most commonly used method, which effectively destroys not only bacteria (1) but also cytomegalovirus (CMV) (2). Moreover, Orloff et al. reported effective inactivation when human immunodeficiency virus type 1 (HIV-1) or HIV-1-infected cells were mixed with human milk prior to pasteurization (3). However, pasteurization may have adverse effects, for example inactivation of biologically active components like immunoglobulins, enzymes, hormones, growth factors, cytokines and heat labile vitamins (1,4,5). It is, however, surprising how little pasteurization seems to affect nutrient bioavailability from human milk, with the exception of fat, the utilization of which seems to correlate to the temperature used. Sterilizing (120°C, 30 min) human milk reduces bioavailable fat by more than 10% because of the formation of surface skin which tends to adhere to the container wall (6), although neither sterilization nor Holder pasteurization impacts on fat content of the milk (6,7). Not unexpectedly, pasteurization affects activities of biologically active proteins in human milk. Notably, both bile salt-stimulated lipase (BSSL) and lipoprotein lipase (LPL) are completely inactivated by Holder pasteurization (1,7,8).

As fat, that is triglycerides (TG) constitutes half of the total energy in human milk and most infant formulae, it serves as the dominating energy substrate for newborn infants. Therefore, efficient digestion and absorption of dietary TG is crucial to infant growth and development. Disregarding this, due to not fully developed exocrine pancreatic and liver functions at birth, fat malabsorption is not uncommon, particularly among preterm newborn infants (9,10). Given similar fat composition in infant formulae as in human milk, fat is more efficiently absorbed from milk than from formulae (10). There are two proposed main reasons for this. The first being presence of BSSL in the human milk, which compensates for low endogenous intraluminal lipase activities during digestion of milk fat (8). The second explanation is the unique structure of human milk TG with 60% of the palmitic acid in the milk being esterified to the *sn*-2 position of TG, which may enhance fat absorption (11–13).

Pasteurized donor milk or own mother's milk is devoid of BSSL activity, but has the same TG structure as raw milk (8). Effects of heat treatment of milk on fat absorption in preterm infants have been evaluated in only a few studies and with conflicting results. Söderhjelm et al. reported surprisingly high coefficient of fat absorption irrespective of whether the milk was pasteurized or not (14). In contrast, Williamson et al., using milk from a single pool of which one third had been pasteurized, found that the Holder pasteurization reduced fat absorption with around 30%, which also corresponded to lower weight gain as compared to feeding the raw milk (15). Some studies have used infants fed mother's own milk as reference group when comparing fat absorption coefficients from different test formulae (10,16). Others have compared mother's own raw milk with pasteurized pooled milk (17). To our knowledge, no study has compared mother's own

milk, raw and pasteurized, with each infant being its own control.

In this study, we addressed the question whether inactivation of BSSL, via Holder pasteurization of own mother's breast milk, reduces absorption of total fat in preterm infants. Furthermore, and most importantly if preterm infants grow faster on raw as compared to pasteurized own mother's milk.

METHODS

Patients and study design

The infants were recruited from patients admitted to the Neonatal Intensive Care Unit of The Queen Silvia Children's Hospital, Gothenburg. Parents were informed and gave consent for their child to participate in the study. Each infant's own mother's milk, raw and pasteurized, respectively was given to five (4 girls and 1 boy) VLBW infants (825–1325 g) with gestational age $\leq$ 30 weeks (27–30 weeks). All infants were on full enteral feeds, free of antibiotics, extra oxygen and had stable weight gain when entering the study. No breastfeeding was allowed during the study period and no fortifiers given. Age at study entry ranged from 9–26 days. The infants were studied for 2 weeks and randomized into two groups of which one started with the pasteurized milk in the first week and continued with raw milk in the second week, while the other was fed the milks in reverse order. During the last 3 days of each week a 72-h fat balance study was performed. To assess growth, weight was registered daily before the first morning meal. Body length, knee–heel length (18) and head circumference was measured at the beginning and at the end of each test week. In order to prevent bias, the investigator assessing growth was blinded with respect to type of feeding. The study was approved by the local ethics committee of Sahlgrenska University Hospital, Gothenburg.

Milk composition and consumption

Each mother participating with her infant in the study collected sufficient volume of milk in advance to meet her infant's needs for the two study weeks, including analysis of macronutrients in the milk. The breast milk was collected at the neonatal ward via a breast pump into a plastic bottle, immediately cooled to 4°C in a water bath located in the refrigerator. After cooling, the milk was pooled and stored at 4°C. Within 8–24 h, the volume estimated to be needed for 24 h had been collected and this volume was now frozen at –20°C until used.

During the test weeks, one plastic bottle with milk intended for 24 h was thawed, heated to 37°C and carefully shaken. Thereafter, triplicate samples of the milk were taken to infrared analysis of the protein, fat and lactose content (19). Prior to each feed, the milk was heated to 37°C. For the test week with pasteurized milk, portions for each 24-h was thawed and pasteurized the day before use. To calculate growth in relation to volume of milk consumed, milk volumes fed for each meal during each test week were noted in the protocol. All meals were given via nasogastric feeding tube and a syringe.

Fat balance

Milk fat

Before the start of each 72-h balance period, the feeding tube was changed. Milk was given every 3-h with a 20-mL disposable syringe via the nasogastric feeding tube and the volume given was noted in the protocol. All spillage and regurgitation were collected on paper napkins, immediately stored at –20°C until analysis and noted in the protocol. Syringes were collected after each meal and the feeding tube retrieved after the last meal and stored at –20°C until analysis.

Milk fat content was analysed by extraction and titration of fatty acids essentially as described by Dole et al. (20).

Stool fat

Carmine red was given as a marker together with the first meal and collecting of stool started with the appearance of first carmine red marker. After 72 h, the second carmine marker was given and stool collection continued until the second carmine marker appeared and this stool was not included. All diapers and paper napkins used during each balance period were collected and stored at –20°C until analysis.

All soiled diapers used during the 72-h balance were saved. The plastic backing and the dry part of the diapers were cut off and the soiled part was weighed to estimate the stool and water content and then placed in a sealed jar. Methanol:chloroform (2:1, vol/vol) was added (10 mL/mg diapers) and lipids were extracted by adding 0.8 volumes of 0.05 M HCL. The mixture was shaken for 30 min and filtered through a filter funnel. To 95 mL of the filtrate, 62.5 mL 0.05 M HCl and 25 mL chloroform was added and the phases were allowed to separate. From the lower chloroform phase 2 × 20 mL was taken to previously weighed dishes. Evaporation was performed for 4 h at room temperature. The mass of lipids were measured gravimetrically. Investigators were blinded to feeding regimens when analysing stool samples. The method for fat extraction used, by adding a chloroform/methanol solvent system to whole solid diapers, has been described and evaluated by Beath et al. (21). Diapers used in the study (SCA Mölnlycke AB, Mölnlycke, Sweden) were found not to contribute fat in the analysis.

Statistics

Data from different test periods were analysed using the non-parametric Wilcoxon Signed Ranks Test with Exact procedure. By using the exact procedure, considerations are taken to the fact that the number of infants is small. p-values <0.05 were considered as statistically significant. The software SPSS v13.0 was used for all calculations.

In the tables, values are expressed both as mean $\pm$ SD and median (range). For milk intake, values are expressed as mean (range).

RESULTS

Milk composition and consumption

The mean (range) milk intake during the week with pasteurized and raw milk was 1619 mL (1193–1917 mL) and 1572 mL

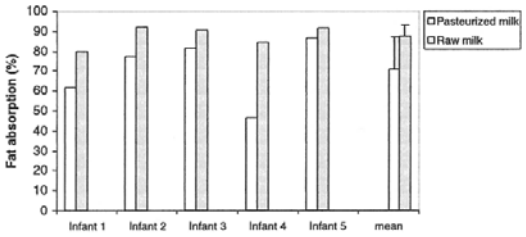

Figure 1 Coefficient of fat absorption in each of the five infants and mean ± SD during the balance with pasteurized and raw milk, respectively.

(1285–2090 mL), respectively. These milk intakes correspond to a mean of 173 mL/kg/24 h when pasteurized milk was given and 175 mL/kg/24 h when the infants received raw milk ($p = 0.625$). These volumes are within current recommendation for preterm infants

There were no statistically significant differences with respect to volume of milk and energy consumed during the 2 weeks studied (Table 1).

Fat balances

There were no significant differences in the amount of total fat consumed during the two 72-h fat balance study periods, although the mean fat intake was slightly higher during the period pasteurized milk was given, that is 23.9 g as compared to 21.3 g when raw milk was given.

For each of the five studied infants, the fat balance with pasteurized milk resulted in higher fat content in the stool as compared with raw milk ($p = 0.063$). The mean net fat absorption coefficient was 17% higher during the balance with raw milk as compared to pasteurized milk, that is 88% (80–92%) versus 71% (47–87%; $p = 0.063$). Individual fat absorption coefficients are shown in Figure 1.

Anthropometric data

On average the infants gained 154 g in weight during the week they were fed raw milk compared to 129 g during the week they were fed pasteurized milk (Table 2). This average difference of 25 g between the two test weeks did not reach statistical significance ($p = 0.188$). This did not change when the weight gain was adjusted for volume of milk consumed and total energy intake ($p = 0.125$). Nor did the difference in length gain reach statistical significance ($p = 0.813$), although the mean gain in length per week was 0.1 cm when the infants received pasteurized milk and 0.4 cm per week when they received raw milk. However, when gain in length was measured as knee–heel length, all five infants in the study increased their knee–heel length more during the week fed with raw milk. The mean increase was 4.16 mm during this week compared to 0.56 mm during the week with pasteurized milk ($p = 0.063$; Table 2). No differences between the two test weeks were found with respect to increase in head circumference (Table 2).

DISCUSSION

In adults, colipase-dependent pancreatic lipase (PTL) is the main enzyme responsible for the digestion of dietary TG. In the newborn infant, and particularly in the preterm infant, exocrine pancreatic functions are not fully developed and the intraluminal PTL activity during established fat digestion is much lower compared to adults (22). In the breastfed infant, the low PTL activity is compensated for by BSSL, which is secreted both from the lactating mammary gland into the milk and the exocrine pancreas. In preterm infants, the milk seems to provide the major part of BSSL in duodenal content during a breast milk meal (23). Besides BSSL, PTL related protein 2 (PLRP2) might have a significant role in dietary fat digestion during the neonatal period (24). Yang et al. reported that the PLRP2 is expressed in exocrine pancreas at birth whereas PTL is not (25). A role of BSSL in neonatal fat digestion is also supported by experiments in mice with disrupted BSSL gene, which showed the consequences of eliminating BSSL from the milk. When BSSL knock out pups were nursed by BSSL knock out dams, this resulted in considerable accumulation of lipid droplets in the distal small intestine because of delayed and inefficient fat digestion and absorption, which was not the case when knock out pups were nursed by wild type dams (26). As heat treatment inactivates BSSL and milk BSSL contributes significantly to the digestive lipase activity in the newborn (1,23), pasteurization of the milk is in a sense a human model partly resembling the knock out mice and can thus be used to study the physiological effects of BSSL on lipid digestion.

Because of the difficulty of recruiting sufficient number of infants for the study, we aimed at including the infants as soon as possible after delivery, when the production of endogenous lipase is low and most of the BSSL activity should originate from the milk.

Table 1 Intake of energy and energy yielding nutrients during the week with pasteurized and raw milk, respectively

	Pasteurized milk		Raw milk		p-value*
	Mean (±SD)	Median (range)	Mean (±SD)	Median (range)	
Volume milk consumed (mL)	1619 (277)	1680 (1193–1917)	1572 (307)	1511 (1285–2090)	0.813
Energy (kcal)	1147 (164)	1153 (900–1353)	1096 (227)	1105 (864–1440)	0.625
Fat (g)	63.5 (8.0)	62.7 (53.4–75.9)	59.4 (14.8)	59.7 (43.6–78.5)	0.625
Protein (g)	22.2 (4.4)	23.4 (14.9–26.0)	23.4 (3.0)	24.2 (19.1–26.5)	0.313
Lactose (g)	110 (19.3)	107.8 (81.3–131.5)	105 (22.5)	103 (85.7–142.9)	0.813

*p-value obtained by using Wilcoxon signed ranks test with exact procedure.

Table 2 Anthropometry during the week fed with pasteurized and raw milk, respectively

Anthropometry	Pasteurized milk		Raw milk		p-value*
	Mean (±SD)	Median (range)	Mean (±SD)	Median (range)	
Weight gain (g/week)	129 (43.65)	140 (60–80)	154 (27.70)	155 (120–190)	0.188
Weight gain (g/week/100 mL milk)	7.76 (1.63)	8.17 (5.03–9.39)	9.98 (2.17)	10.03 (7.94–13.31)	0.125
Weight gain (g/week/100 kcal)	10.97 (2.54)	11.64 (6.67–13.30)	14.50 (3.96)	14.02 (10.42–20.62)	0.125
Length gain (cm/week)	0.1 (0.42)	0.0 (−0.5 to 0.5)	0.4 (1.08)	0.0 (−0.5 to 2.0)	0.813
Knee–heel gain (mm/week)	0.56 (2.05)	0.60 (−2.6 to 2.7)	4.16 (1.28)	4.50 (2.40–5.80)	0.063
Head circumference gain (cm/week)	0.88 (0.18)	1.00 (0.60–1.00)	0.86 (0.27)	0.80 (0.60–1.30)	1.000

*p-value obtained by using Wilcoxon Signed Ranks Test with Exact procedure.

Further, by using each infant's own mother's milk and each infant as its own control in a cross-over design, we increased the chance of observing a difference even with a small number of infants. In fact, we did find higher coefficient of fat absorption (border line significance) from raw as compared to pasteurized milk, although the mean difference (17%) was less than the one-third reported by Williamson et al. (15). However, when calculating absorption coefficients based on grams of fat/kg/day, the respective figures were 73.6% and 53.7% from raw and pasteurized milk, respectively (15), a difference of 19.9%, which is close to the 17% difference we found using the same type of calculation. The difference may in fact be due to a difference in fat intake. The mean milk intake of raw and pasteurized milk was 265 mL/kg/day and 255 mL/kg/day, respectively in the former study compared to 175 mL/kg/day and 173 mL/kg/day in our study. Assuming the same fat content, a higher fat intake may have reduced the coefficient of fat absorption from raw milk which in turn may explain a larger effect of inactivation of BSSL in their study. High fat intake, leading to malabsorption, may explain why the infant with the greatest fat intake also had the poorest fat absorption coefficient in our study. An inverse relation between fat intake and coefficient of absorption was also noted in the Williamson et al.'s study (15). The mean fat absorption of 88% from raw milk in the present study is identical to what Atkinson et al. found in a study on preterm infants fed with mother's own milk (16). These authors also performed fat balance with pooled milk heated to 100°C for 5 min, which further reduced fat absorption to 64%. A likely reason for the lower fat absorption compared to our results (71%) is the high temperature used, which may affect accessible fat due to adherence to the container wall (6), and possibly the use of pooled breast milk collected from mothers during the second to fifth month post-partum (16). Williamson et al. also found that higher temperature than Holder pasteurization further decreased fat absorption (15), that is from 53.7% with pasteurized milk to 45.9% with boiled milk. The coefficient of fat absorption in our study is also very similar to that reported by Chapell et al. (10) and Carnielli et al. (9).

Interestingly, higher fat absorption from raw milk was accompanied by more rapid linear growth assessed by knee–heel length, which is a more sensitive measure of linear growth than body length (18). All infants increased their knee–heel length more during the week they were fed raw milk, also when adjusted for volume of milk consumed.

Four of the five infants also gained more weight when fed raw as compared to pasteurized milk. In the fifth infant, we noted 10 g lower weight gain during the period with raw milk. The explanation may be that this infant became ill the last day of the first test week (pasteurized milk) and treated with diuretics from that day throughout the following week when fed raw milk. In agreement with our observation, Stein et al. found that the preterm infants reached birth weight sooner when fed raw own mother's milk as compared to Holder pasteurized pooled breast milk (17). While the infants in our study on an average gained 9.98 g/100 mL when fed raw milk, the corresponding weight gain was 9.24 g/100 mL in the Williamson et al. (15) and from pasteurized milk the respective figures were 7.76 g/100 mL and 6.34 g/100 mL. Although infants in the latter study had higher milk intake (mL/kg/day) compared to the infants in our study, they had slightly lower weight gain (g/100 mL), supporting the notion that the increasing fat intake results in decreasing coefficient of fat absorption, which in turn may affect weight gain.

Although the differences between the two study weeks in fat absorption and knee–heel length gain only reach border line significance, given the low number of infants we believe that our results showed that the pasteurization of mother's own milk has an effect on preterm infant's fat absorption and growth.

In conclusion, we found lower coefficient of fat absorption from pasteurized as compared to raw own mother's milk. Moreover, infants gained more in weight and also in length when fed raw as compared to pasteurized milk. Using knee–heel length as assessed by the sensitive knemometer, we did in fact detect greater gain in linear growth during the week with raw milk. These observations should be considered when decisions are made on pasteurization of donor breast milk and pasteurization of own mother's milk to prevent transmission of CMV via the breast milk. In the future, a possibility to circumvent this problem might be to supplement pasteurized milk with recombinant human milk BSSL (27), thus restoring the endodgenous lipolytic activity of the milk.

ACKNOWLEDGEMENT

The authors wish to thank the staff at the Neonatal Intensive Care Unit of The Queen Silvia Children's Hospital, Gothenburg for all the careful work and Miss Eva-Lotta Vestman for skilful work with the fat extraction. For statistical advice, we are grateful to Assistant Prof. Hans Stenlund.

The study was financially supported by the Swedish Research Council, Medicine, Her Royal Highness the Crown Princess Lovisa's Society for Pediatric Research/Axel Thielman's Memorial Fund, Västerbotten County Council and Astra Hässle AB.

References

1. Björkstén BBLG, De Chateau P, Fredrikzom B, Gothefors L, Hernell O. Collecting and banking: to heat or not to heat? *Br Med J* 1980; 281: 765–76.
2. Hamprecht K, Maschmann J, Muller D, Dietz K, Beseuthal J, Goelz R, et al. Cytomegalovirus (CMV) inactivation in breast milk: reassessment of pasteurization and freeze-thawing. *Pediatr Res* 2004; 56: 529–35.
3. Orloff SL, Wallingford JC, McDougal JS. Inactivation of human immunodeficiency virus type I in human milk: effects of intrinsic factors in human milk and of pasteurization. *J Hum Lact* 1993; 9: 13–7.
4. Lawrence RA. Storage of human milk and the influence of procedures on immunological components of human milk. *Acta Paediatr Suppl* 1999; 88: 14–8.
5. Tully DB, Jones F, Tully MR. Donor milk: what's in it and what's not. *J Hum Lact* 2001; 17: 152–5.
6. Fidler N, Sauerwald TU, Koletzko B, Demmelmair H. Effects of human milk pasteurization and sterilization on available fat content and fatty acid composition. *J Pediatr Gastroenterol Nutr* 1998; 27: 317–22.
7. Henderson TR, Fay T, Hamosh M. Effect of pasteurization on long chain polyunsaturated fatty acid levels and enzyme activities of human milk. *J Pediatr* 1998; 132: 876–8.
8. Hernell O. Human milk lipases III. Physiological implications of the bile salt stimulated lipase. *Eur J Clin Invest* 1975; 5: 267–72.
9. Carnielli VP, Verlato G, Pederzini F, Luijendij K, Boerlage A, Pedrotti D, et al. Intestinal absorption of long-chain polyunsaturated fatty acids in preterm infants fed breast milk or formula. *Am J Clin Nutr* 1998; 67: 97–103.
10. Chappell JE, Clandinin MT, Kearney-Volpe C, Reichman B, Swyer PW. Fatty acid balance studies in premature infants fed human milk or formula: effect of calcium supplementation. *J Pediatr* 1986; 108: 439–47.
11. Martin JC, Bougnoux P, Antoine JM, Lanson M, Couet C. Triacylglycerol structure of human colostrum and mature milk. *Lipids* 1993; 28: 637–43.
12. Lucas A, Quinlan P, Abrams S, Ryan S, Meah S, Lucas PJ. Randomised controlled trial of a synthetic triglyceride milk formula for preterm infants. *Arch Dis Child Fetal Neonatal Ed* 1997; 77: F178–84.
13. Carnielli VP, Luijendijk IH, Van Goudoever JB, Suikers EJ, Boerlege AA, Degenhart HJ, et al. Structural position and amount of palmitic acid in infant formulas: effects on fat, fatty acid, and mineral balance. *J Pediatr Gastroenterol Nutr* 1996; 23: 553–60.
14. Söderhjelm L. Fat absorption studies in children influence of heat treatment on milk on fat retention by premature infants. *Acta Paediatr* 1952; 41: 207–21.
15. Williamson S, Finucane E, Ellis H, Gamsu HR. Effect of heat treatment of human milk on absorption of nitrogen, fat, sodium, calcium, and phosphorus by preterm infants. *Arch Dis Child* 1978; 53: 555–63.
16. Atkinson SA, Bryan MH, Anderson GH. Human milk feeding in premature infants: protein, fat, and carbohydrate balances in the first two weeks of life. *J Pediatr* 1981; 99: 617–24.
17. Stein H, Cohen D, Herman AAB, Rissik J, Ellis U, Boltou K, et al. Pooled pasteurized breast milk and untreated own mother's milk in feeding of very low birth weight babies: a randomized controlled trial. *J Pediatr Gastroenterol Nutr* 1986; 5: 242–7.
18. Michaelsen KF, Skov L, Badsberg JH, Jorgensen M. Short-term measurement of linear growth in preterm infants: validation of a hand-held knemometer. *Pediatr Res* 1991; 30: 464–8.
19. Michaelsen KF, Pedersen SB, Skafte L, Jaeger P, Peitersen B. Infrared analysis for determining macronutrients in human milk. *J Pediatr Gastroenterol Nutr* 1988; 7: 229–35.
20. Dole VP, Meinertz H. Microdetermination of long-chain fatty acids in plasma and tissues. *J Biol Chem* 1960; 235: 2595–9.
21. Beath SV, Willis KD, Hooley IM, Brown GA, Kelly DA, Booth IW. New method for determining faecal fat excretion in infancy. *Arch Dis Child* 1993; 69: 138–40.
22. Fredrikzon BH, Hernell O, Olivecrona T. Decrease of lipase and esterase activities in intestinal contents of newborn infants during test meals. *Pediatr Res* 1978; 12: 631–4.
23. Fredrikzon BHO, Bläckberg L, Olivecrona T. Bile salt-stimulated lipase in human milk: evidence of activity in vivo and of a role in the digestion of milk retinol esters. *Pediatr Res* 1978; 12: 1048–52.
24. Li X, Lindquist S, Lowe M, Noppa L, Hernell O. The bile salt-stimulated lipase and pancreatic lipase-related protein 2 are the dominating lipases in neonatal fat digestion in mice and rats. *Pediatr Res* (in press).
25. Yang Y, Sanchez D, Figarella C, Lowe ME. Discoordinate expression of pancreatic lipase and two related proteins in the human fetal pancreas. *Pediatr Res* 2000; 47: 184–8.
26. Howles PN, Stemmerman GN, Fenoglio-Preiser CM, Hui DY. Carboxyl ester lipase activity in milk prevents fat-derived intestinal injury in neonatal mice. *Am J Physiol* 1999; 277: G653–61.
27. Strandvik BH, Hernell O. Recombinant human milk bile salt stimulated lipse improves lipid uptake and reduces the pancreatic supplementation in patients with cystic fibrosis. *Pediatr Pulmonol* 2004; Suppl. 27: 333 (abstract).